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MALE-SPECIFIC STRUCTURES ON THE WINGS OF THE GULF FRITILLARY BUTTERFLY, *AGRAULIS VANILLAE* (NYMPHALIDAE)

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ABSTRACT. Males of the Gulf Fritillary butterfly, *Agraulis vanillae* Linnaeus (Nymphalidae), have distinctive structures on certain veins of the dorsal wing surface that appear to be involved in pheromone production. Here we confirm and extend an earlier description of these structures. Observations using light and scanning electron microscopy indicate that in these structures, patches of several scale types alternate with scaleless areas along the veins. Some of these open areas have pores that we suggest might be the route by which the pheromone moves from cells in the wing integument onto brush-bearing scales from which it is disseminated during courtship. We have also found that although the dimensions of the basic units of these structures on the veins are not correlated with body size, larger males do have greater total vein length devoted to these structures. These findings are discussed in light of the courtship of this species and the potential for these structures to be involved in mate choice and to be a product of sexual selection.

Additional key words: pheromones, androconia, morphology, mating behavior, SEM.

In many butterflies and moths, males have structures on their wings or body that produce chemical signals, or pheromones, used by males when courting females (Myers & Brower 1969, Pliske & Eisner 1969, Vane-Wright & Boppré 1993, Kan & Hidaka 1997, Iyengar et al. 2001). In some cases, behavioral experiments have confirmed the function of these structures, but more often a phermonal function is inferred from the high surface area of all parts of them, and the observation that they are often brought close to the antennae of the female during courtship (Lundgren & Bergström 1975, Rutowski 1977, Boppré 1984). Although much is known of the morphology and operation of such structures in danaine butterflies, the full diversity of their structure and function in butterflies is generally not well documented.

Müller (1877) first discovered and described presumptive scent-producing structures on the wings of males of the Gulf Fritillary, *Agraulis vanillae* Linnaeus (Nymphalidae), a common heliconiine butterfly in the American tropics and subtropics. In particular, he reported that along six of the forewing (FW) veins there were patches of long thin scales each bearing a brush at the distal end, which alternated with rows of normal scales crossing the vein. Our preliminary observations of these structures using light microscopy suggested that the arrangement of scales along these veins while serially repeated, as Müller (1877) reported, was more complex than indicated by his description and so we undertook a more detailed study of their morphology using light and scanning electron microscopy.

Our first objective was to repeat and extend Müller's observations because we believed more detailed observations could suggest how the scent was produced and disseminated in *A. vanillae*. The second objective was to determine how much, if any, inter-individual variation there is in the overall size of these structures. This could suggest the extent to which males might vary in their ability to produce critical chemical signals during courtship. Lastly, we wanted to relate Müller's and our observations to published descriptions of the courtship behavior of *A. vanillae* (Rutowski & Schaefer 1984).

METHODS

We examined the FWs of male and female *A. vanillae* using both light and scanning electron microscopy. Specimens were either laboratory-reared from field-collected larvae and eggs on cuttings of *Passiflora* spp. or field-caught as adults in Tempe, Arizona. For examination of scale morphology, we chose butterflies with little or no wing wear. In some cases, we removed scales with a small paintbrush to expose the cuticle covering the veins underlying the scales. For light microscopy we mounted FWs onto slides and photographed specialized structures and scales. For scanning electron microscopy (SEM), we attached wings to stubs with conducting paint and no coating, and observed them with a Philips/FEI XL 20 scanning electron microscope.

To determine whether larger males also have a larger androconial area on their FWs, and thus greater pheromone-disseminating ability, we measured the length of the portion of each of the veins containing specialized structures on the male FW. For each specimen (n = 20), we then totaled these lengths and plotted this total against FW length and subjected the data to correlation analysis. We also determined the density of specialized structures along the veins to investigate whether it also varies with FW size. To do this we estimated the number of structures per mm on each of the six veins on a male FW. We then calculated the

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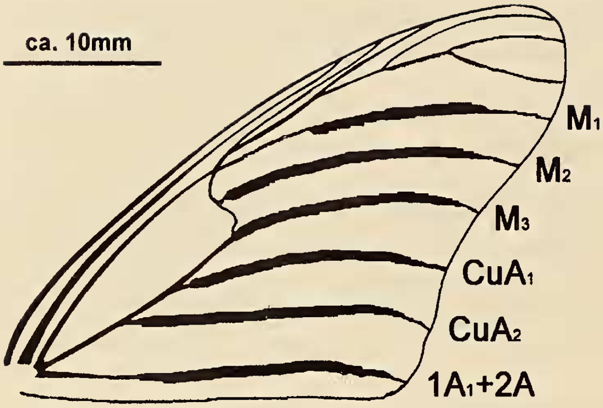

FIG. 1. A sketch of the dorsal side of the FW of the male Gulf Fritillary butterfly with the veins containing the presumptive pheromone-disseminating structures labeled according to Scoble's (1992) notation. The segments of the veins containing the specialized structures are highlighted.

mean number of structures per mm for all 6 veins and compared the mean number of structures per mm to the FW length, also using correlation analysis (n = 20).

RESULTS

As reported by Müller (1877), male-specific scales and other structures are found on the dorsal FW of the male on veins M_1, M_2, M_3, CuA_1, CuA_2, and $1A_1$ + 2A (Fig. 1; wing vein notation, Scoble 1992). On veins that had not been disturbed we observed that, along at least part of each of the six veins, rows of brush-like scales alternated with rows of scales with broad, straight edges (Fig. 2). The average length of each vein segment that contains the male-specific structures is 11.7 ± 1.43 mm on M_1, 13.4 ± 1.51 mm on M_2, 13 ± 1.57 mm on M_3, 13.5 ± 1.69 mm on CuA_1, 14.7 ± 1.74 mm on CuA_2 and 12.96 ± 1.73 mm on $1A_1$ + 2A. The FW length of the 20 specimens we examined averaged 33.7 ± 2.6 mm.

With the scales removed from the veins, we found that a set of five distinct areas make up a unit 0.24 mm in length that is serially repeated along each of the six veins (Fig. 3). In order from the wing base to the wing edge, the five distinct areas are the inter-scale vein-section 1 (IVS-1), the scale-section 1 (SS-1), the inter-scale section 2 (IVS-2), the scale-section 2 (SS-2) and the porous section (PS) (Figs. 3, 4).

As shown in Fig. 4, the IVS-1 (mean length = 0.12 mm) occurs between the repetitive structures and does not contain any scale attachment sites. Every third row of scales occuring on the inter-vein part of the wing runs uninterrupted across the veins. SS-1 (mean length = 0.02 mm) is the scale attachment row after IVS-1 and contains two different types of scales.

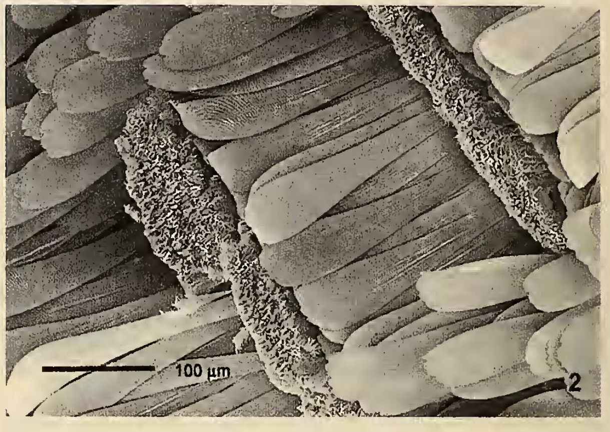

FIG. 2. An SEM of a segment of the vein containing the specialized structures with the scales intact (250×). The brush-like scent scales are attached to the vein at SS-2 and the broad flat scales are attached at SS-1.

One type is broad at the top, narrow at the base and shiny black, whereas the other type is equally as long, but broad at the base and tip and translucent, shiny gold. Both types of scales are flat and the black scales fit snugly over the gold, scales as shown in Fig. 5. IVS-2 (mean length = 0.01 mm) is the area containing no scale attachments between SS-1 and SS-2. SS-2 (mean length = 0.04 mm) is a region with the scale attachments between IVS-2 and PS. The scales arising in SS-2 are densely packed, long (mean length = 0.4 mm), narrow, and have brush-like ends with high surface area. In addition, the bases of these scales are black, followed by a thin transparent area, then by a black, narrow section which contracts into another thin transparent area, followed by a black brush-like apex (Fig. 6). These brush-bearing scales are positioned so that the transparent basal area (mean length = 0.04 mm) overlies PS as shown in Fig. 6. PS (mean length = 0.06

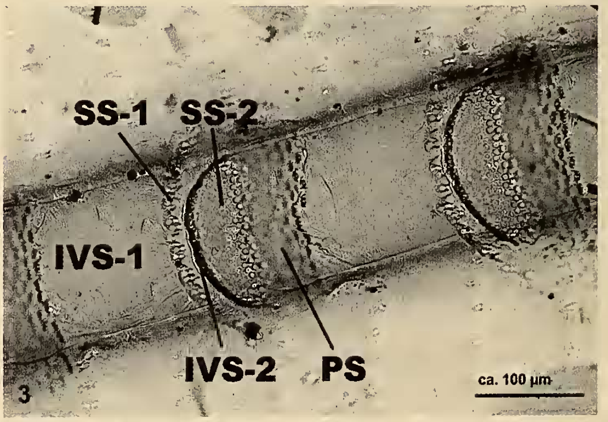

FIG. 3. The repetitive nature of the specialized structures along the vein of a male's FW is shown with the scales removed (20×). The five distinct sections of the specialized structures are labeled according to their function: Scale Section 1 and 2: SS-1 and SS-2; Interscale Vein Section 1 and 2: IVS-1 and IVS-2; and Porous Section: PS.

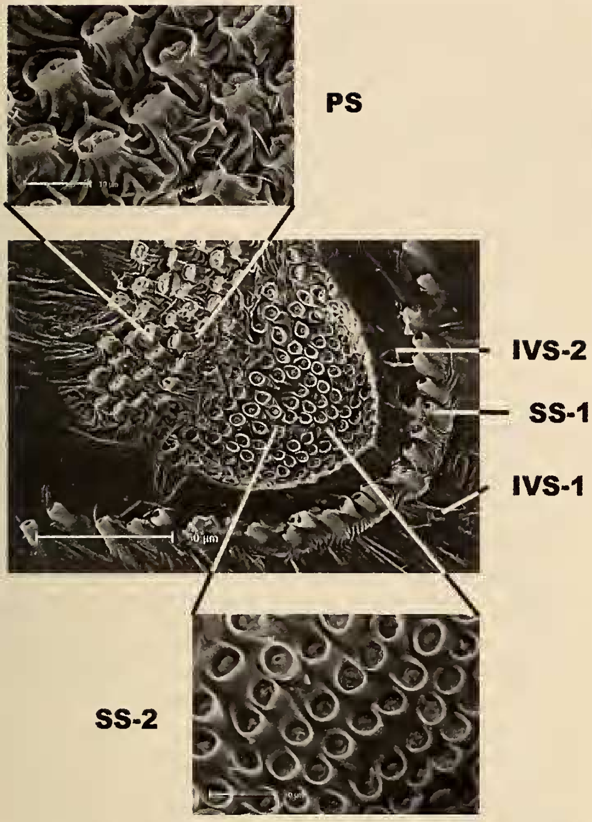

FIG. 4. An SEM of the male Gulf Fritillary pheromone-disseminating structure with the five distinct sections labeled (750×). PS and SS-2 are further magnified in order to clearly see the difference between the two areas (1500×). SS-2 is the scale attachment point for the scent scales and PS is the hypothesized area of pheromone secretion.

mm) is the porous area containing no scale attachment sites adjacent to SS-2.

The total length of the vein segments that contain the presumptive pheromone-disseminating structures is positively correlated with FW length ($r^2 = 0.858$, $p < 0.0001$) (Fig. 7). This means that the longer the FW of the male, the longer the length of the veins containing the pheromone-disseminating structures. However, there is no relationship between the mean number of structures per mm and the length of the FW ($p > 0.40$) (Fig. 8). Therefore, the number of structures per mm along the veins remains constant regardless of the size of the FW. Collectively, these results indicate that males with longer FWs have more total scent-disseminating structures than males with shorter FWs and thus greater scent disseminating ability.

DISCUSSION

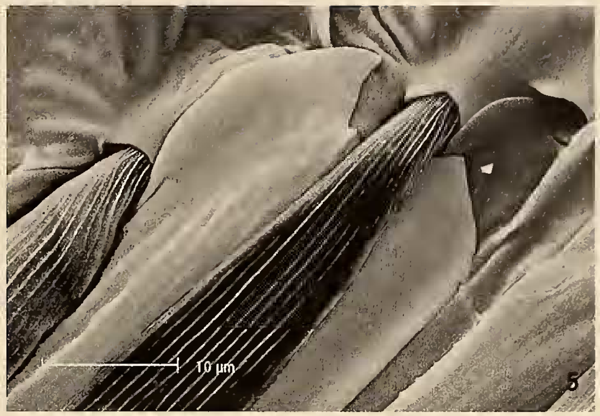

FIG. 5. An SEM of the two types of scales and their attachment points at SS-1 (1500×). Both scale types are flat and one fits snuggly over the other. The scale type that lies on top is broad at the top, narrow at the base and shiny black. The other scale type is equally as long, broad at the base and tip and translucent, shiny gold in color.

Our observations confirm and extend Müller's (1877) description of male-specific structures found along certain veins on the dorsal FW surface of *A. vanillae*. In addition, we found that these male-specific structures consist of serially repeated units of scale arrangements along the veins. However, each unit consists of five rather than two distinct elements, as described by Müller. One of these elements is a patch of brush-bearing scales and rows of scales typical of those found crossing the vein elsewhere on the wing (Magnus 1950). We also found other scale types and three regions without scales that included an area dotted with large pores opening onto the wing surface.

The specific elements of the structures found along these veins may interact in the following ways to disseminate a pheromone. The brush-bearing scales located in SS-2 lie directly over the porous area (PS) (Fig. 6). These pores could be the openings through which the product of pheromone-producing cells in

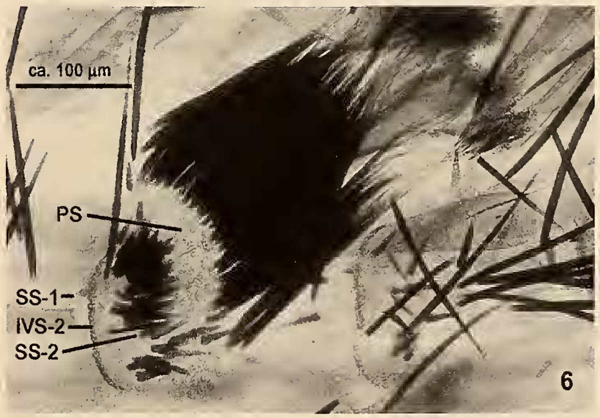

FIG. 6. A photograph of a pheromone-disseminating structure on the male FW with the scent scales intact at SS-2 (20×). The transparent portion of the scent scales closest to the point of attachment lies directly over the porous area (PS). PS is hypothesized to secrete pheromones that disseminate onto the high surface area brush-like scales.

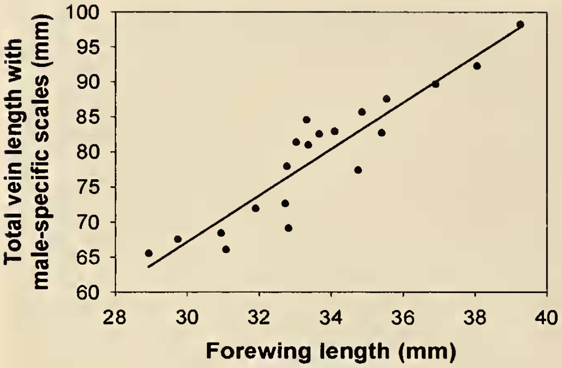

FIG. 7. The positive relationship between the FW length and the total length of the vein segments containing pheromone-disseminating structures ($r^2 = 0.858$, $p < 0.001$).

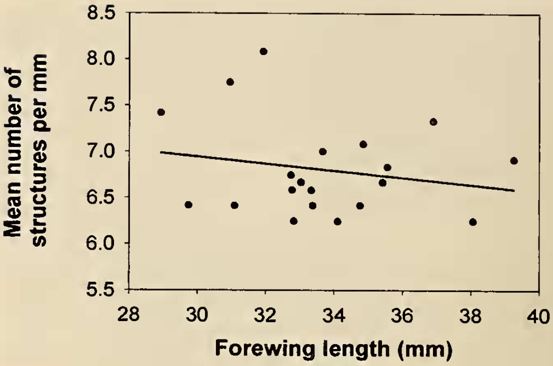

FIG. 8. No relationship exists between the number of structures per mm per individual and the FW length. In other words, the density of androconia along the veins remains constant regardless of FW size ($p > 0.40$).

the wing integument is secreted. This arrangement would allow the compounds to be directly transferred onto the densely packed, high surface area brushes on the overlying scales. The black and gold scales described in SS-1 lie over the narrow stalk of delicate scent-scales (Fig. 2). These SS-1 scales, which were difficult to remove, appear to form a protective barrier over the relatively fragile and easily removable SS-2 brush-bearing scales. This protective barrier may help prevent the loss of brush-bearing scales and excessive evaporation of pheromone secretions.

Rutowski and Schaefer (1984) described the courtship behavior of *A. vanillae* and reported a male display, which they called the wing clap. During courtship the male positions himself head-to-head alongside the female with his body at about a 45° angle relative to the body of the female. Initially his wings are slightly spread and the female's ipsilateral antenna comes to lay back between the male's wings. He then quickly closes and reopens his wings repeatedly catching the female's antenna between them and bringing the female antenna into brief contact with the distinctive structures on the dorsal FW veins. Hence, as in other species, we find a male courtship behavior that strongly suggests that these male-specific, high surface area structures are producing a pheromone that may be important in mate choice by females (Tinbergen et al. 1942, Magnus 1950, Brower et al. 1965, Conner et al. 1980, Pivnick et al. 1992).

Our data show that males with longer FWs have a greater total length of veins containing pheromone-disseminating structures (Fig. 7). Because the density of androconia per individual is not affected by FW length (Fig. 8), and FW length is a good measure of male size, larger males should have a greater total number of pheromone-disseminating structures than smaller males. Thus, the number of pheromone-disseminating structures and the quantity of pheromone secreted are positively correlated with male size. A similar correlation was found in the moth *Utetheisa ornatrix* and was determined to be a heritable trait (Iyengar & Eisner 1999). If the amount of pheromone disseminated by the male during courtship is critical to female mate choice in *A. vanillae*, larger males should be more successful at mating than smaller males. This is the case in *U. ornatrix* where the amount of pheromone disseminated by the male is the only criterion used by the female when choosing a mate (Iyengar et al. 2001). Similarly, males in *Drosophila grimshawi* deposit a pheromone to attract females to a mating site by rubbing their abdomen on the substrate. Males who deposit the greatest amount of pheromone at their site are the most successful at attracting females and mating (Droney & Hock 1998).

The evidence discussed above indicates that the morphology and location of the male androconia in *A. vanillae* may be a product of sexual selection (Fisher 1958, Baker & Cardé 1979, Eisner & Meinwald 1995). The quantity of the pheromone secreted may be an indicator of male quality that females evaluate when choosing a mate (Dussourd et al. 1991, LaMunyon 1997, Iyengar & Eisner 1999). Quality here could mean species identity or ability to provide either material (i.e., defensive secretions) or genetic benefits (i.e., genes for large size). Pheromone quantity may also indicate a male's age and/or mating status because scent-scales are likely to be lost with age and with each mating or courtship. In this way a female may be able to assess the physiological state of a male when choosing a mate.

However, sometimes the Gulf Fritillary female will mate with a male even when he does not perform the wing-clap display (Rutowski & Schaefer 1984). Perhaps pheromones are not the only indicator of a male's quality, female choice is not based on this character, some females are less discriminating than others, or some males have a strong enough odor to elicit a receptive response from the female without using the display. To better understand the functions of these pheromone-disseminating structures, further investigation needs to be undertaken to identify the pheromones, to determine which of these pheromones are behaviorally active during courtship and mating, and whether pheromone amount affects female mate choice.

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